affects donor T cell expansion, activation and migration. We compared lethally irradiated vs. non-irradiated Balb/c (H-2^d) mice that received allogeneic luciferase$^+$ FVB/N T cells (H-2^q). *In vivo* bioluminescence imaging (BLI) visualized the kinetics of aGVHD and guided more detailed analyses. Irradiated wild type Balb/c, Balb/c Rag$^{-/-}$ and Balb/c Rag$^{-/-}$cychain$^{-/-}$ aHCT recipients displayed comparable kinetics of aGVHD initiation in lymphoid organs until day +3 and started intestinal infiltration by day +4, skin and liver infiltration by day +5-6. All mice succumbed to lethal aGVHD within two weeks after aHCT. Non-irradiated Balb/c wild type recipients did not develop aGVHD. BLI revealed the process of graft rejection after initial signal increase over lymphoid organs by day +3. In contrast donor cells overcame host resistance in non-irradiated Balb/c Rag$^{-/-}$ and Balb/c Rag$^{-/-}$cychain$^{-/-}$ mice. However, despite proliferation in lymphoid organs, infiltration of aGVHD target tissues was delayed. Balb/c Rag$^{-/-}$ displayed moderate and Balb/c Rag$^{-/-}$cychain$^{-/-}$ mice even weaker intestinal infiltration on day +6. FACS analysis revealed that donor T cell subsets were less activated in non-irradiated as compared to irradiated hosts. Non-irradiated Balb/c Rag$^{-/-}$cychain$^{-/-}$ mice succumbed to lethal aGVHD within two months after aHCT. Balb/c Rag$^{-/-}$ mice did not die from aGVHD suggesting that host NK cells controlled alloreactive T cells. In summary host conditioning exacerbates aGVHD by increased alloreactive T cell activation and recruitment to aGVHD target tissues.

145

EPIDERMAL DENDRITIC CELL DEPLETION WITH MYELOABLATIVE CONDITIONING THAT DOES NOT RECOVER POST TRANSPLANT

Hofmeister, C.C.[1], Peterson, A.[1], Tara, K.[2], Stiff, P.[1], Curry, J.[1], Nickoloff, B.J.[1] 1. Loyola Cardinal Bernardin Cancer Center, Maywood, IL; 2. Lake Erie College of Osteopathic Medicine, Bradenton, FL.

Introduction: Cutaneous graft-versus-host disease (GVHD) is an antigen presenting cell mediated process which leads donor T lymphocytes to presumably target keratinocyte progenitor cells at the dermal-epidermal junction. In the absence of inflammation, epidermal dendritic cells (a.k.a. Langerhans cells or LCs) can self-renew for long periods of time without the requirement for a bone-marrow derived blood-borne precursor. Murine models have suggested that depletion of host LCs pretransplant prevents cutaneous GVHD. We sought to determine epidermal dendritic cell density pre-transplant, post-transplant, and with the onset of acute GVHD. **Methods:** We reviewed all skin biopsies performed on 2101 stem cell transplants patients at Loyola University Medical Center from 1995-2005. From these paraffin-embedded blocks, 20 randomly selected acute GVHD biopsies, all 17 biopsies of non-GVHD rashes in post allograft patients, and 12 normal skin biopsies from reduction mammoplasties were obtained. In addition as part of a prospective trial, 14 patients underwent punch biopsies from December 2004 to August 2005 of grossly normal skin before and immediately following the completion of their conditioning regimens. All biopsies were stained for CD1a (LC marker) and the number of suprabasal stained cells per high power field (HPF) were counted by a dermatopathologist. **Results:** Total body irradiation containing (TBI) conditioning regimens nearly completely eliminated epidermal LCs by the day of transplant. In comparison to normal skin (44 CD1a+ cells ± 4.7 [95% CI]), acute GVHD lesions showed a depletion of epidermal LCs (3.7 CD1a+ cells ± 3.2 [95% CI]), but so did biopsies obtained post allograft without GVHD (6.5 CD1a+ cells ± 3.6 [95% CI]). **Conclusions:** Myeloablative conditioning regimens deplete epidermal LCs and this reduction may remain post engraftment. UV dermatitis, topical steroids or tacrolimus, viral infections (HPV, HIV), and GVHD all induce LC depletion. The mechanism for this depletion is unknown but mediators such as TNF-α and IL-1 have induced LC migration in murine models (Roake JE, *J Exp Med*, 1995). Our data suggest that pretransplant LC depletion in humans will not prevent cutaneous GVHD. Future studies will need to be performed to determine the origin of LCs in post allograft patients.

146

HOST LANGERHANS CELLS (LCs) CAN BE THERAPEUTICALLY MANIPULATED *IN VIVO* WITH IMIQUIMOD (TLR7 AGONIST) TO AUGMENT DLI-MEDIATED GVH AND GVL REACTIVITY

Skarica, M., Radojcic, V., Luznik, L. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD.

We recently found in murine models that after MHC-matched allografting, the residual host LCs, the major dendritic cells (DCs) of the skin, survive in epidermis despite the presence of large numbers of peripheral donor T cells in the graft and conversion to the full donor DC chimerism in blood. This observation led us to hypothesize that *in vivo* manipulation of residual host LCs, which persist in complete donor chimeras after MHC-matched allografting, may have a central role in augmenting DLI-mediated alloimmune responses. We tested our hypothesis in two murine models of MHC-matched allografting. To manipulate T cell-LC interaction *in vivo* we used the Toll-like receptor 7 (TLR7) ligand imiquimod. Topical application of imiquimod is known to augment *in situ* maturation of the LCs and enhance their emigration from the skin to the skin-draining lymph nodes (LNs). We first tracked the *in vivo* fate of DLI-derived T cells after their administration to the 8-week-old B6.SJL→C3H.SW complete donor chimeras that were pretreated with vehicle or imiquimod. As DLI, we used purified donor T cells from the B6.PL-*Thy1^a* mice that differ in the expression of Thy 1.1 allele. In the imiquimod treated group, the expansion of DLI-derived Thy 1.1$^+$ T cells in LNs and spleen was significantly better than that of the vehicle-treated group. This augmented DLI-mediated GVH response was also reflected by a higher number of DLI-derived CD8$^+$ INF-γ secreting T cells and by an increase in donor-derived LC chimerism in the imiquimod-treated group. Next, we tested the effect of imiquimod on the GVL reactivity of DLI. Four-week-old C3H.SW→C57/BL6 chimeras constructed after lethal conditioning were pretreated with imiquimod prior to DLI administration and lethal challenge with C1498 leukemia cells. Chimeras that received imiquimod and DLI had superior leukemia-free survival in comparison to animals that received DLI plus vehicle ($P < .01$) or imiquimod alone ($P < .02$). The superior leukemia-free survival in the group that received DLI plus imiquimod correlated also with faster conversion to full donor CD8$^+$ T cell chimerism in comparison to the DLI plus vehicle group ($P < .01$). In both models, we have not observed any significant clinical signs of GVHD. These results indicate that imiquimod, through its action on LCs, can be used to enhance the DLI-mediated alloimmune responses including their GVL reactivity without exacerbating GVHD.

147

SIGNIFICANT DECREASE IN CORD BLOOD (CB) VERSUS ADULT PERIPHERAL BLOOD (APB) MONOCYTE (MO)–DERIVED DENDRITIC CELL (DC) GENE AND PROTEIN EXPRESSION PATTERNS AND T CELL FUNCTIONAL ACTIVATION: INSIGHT INTO IMMATURITY OF CB CELLULAR IMMUNITY

Jiang, H.[1], Wade-Harris, C.[1], Baxi, L.[2], Cairo, M.S.[1,3,4] 1. Department of Pediatrics, Columbia University, New York, NY; 2. Department of Obstetrics & Gynecology, Columbia University, New York, NY; 3. Department of Medicine, Columbia University, New York, NY; 4. Department of Pathology, Columbia University, New York, NY.

It has been recognized that dysfunction of CB cellular immunity is in part due to the immaturity of the CB cellular immune system (Cairo, 1997). However, biological pathways and molecular mechanisms associated with the immaturity of CB cellular immunity are still poorly understood. Recently we have utilized oligonucleotide microarray to examine gene expression profile of CB vs APB Mo and have demonstrated significant differential gene expression patterns (Jiang/Cairo, 2004). In the current study, differential expressed genes and proteins were examined in Mo-derived CB vs APB DC by means of oligonucleotide microarray and proteomics. Briefly, Mo were purified and cultured for 8 days with GM-CSF, IL-4 and LPS. Oligonucleotide microarray was carried out (Affymetrix). The proteomic study was conducted by liquid chromatography (LC) and tandem mass spectrometry (MS/MS). We identified gene expression patterns that were significantly lower in CB